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Research Article

IN VITRO ANTIOXIDANT PROPERTIES OF *NOTHAPODYTES NIMMONIANA* (Grah.) Mabb. (ICACINACEAAE)

UMA.G*, JAGATHES KUMAR. S AND BALASUBRAMANIAM.V

PG and Research Department of Botany,Kongunadu Arts And Science College(Autonomous), Coimbatore-29,Tamil Nadu,India,
Email: umagraj89@gmail.com.

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ABSTRACT

This study was designed to examine the in vitro antioxidant activities of the methanolic extracts of leaf and stem of *Nothapodytes nimmoniana*(Grab.)Mabb. belongs to the family Icacinaceae. Antioxidant activity of methanolic extracts were determined using various *in vitro* models like DPPH (1,1-diphenyl-2-picryl hydrazine) $ABTS^{\bullet+}$ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) Ferrous iron chelating ability, Reducing power. In the above four assays, methanolic extracts showed antioxidant potential to varying degrees. Stem showed maximum antioxidant activity of 84.35% to 250µg/ml by DPPH method as compared to the standard. It was concluded that the methanolic extracts of different parts of *N. nimmoniana* showed significant antioxidant activity.

Keywords: *Nothapodytes nimmoniana*, ABTs, DPPH, Ferrous iron-Chelating ability, Reducing power

INTRODUCTION

India is a home of traditional medicine systems which possess large extent of higher plants approximately 4,00,000 species in the world as compared to animal's species that are about 5-10 million[3]. About 80% of world population utilizes plant as their primary source of medicinal agents[12]. Antioxidant supplements or foods rich in medicinal plants may be used to help the human body in reducing oxidative damage by free radicals and active oxygen[4]. Therefore currently, the research interest is focused on the potential role of antioxidants. Plants are a good source of biologically active compounds known as phytochemicals. The phytochemicals have been found to act as antioxidants by scavenging free radicals, and many have therapeutic potential for free radical associated disorders[7,9] . It is well known that free radicals are the major cause of various chronic and degenerative diseases such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer[11]. There fore, it is important to assess antioxidant activity of the plants used in the herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on antioxidant activity of these plants.

Plants of the genus *Icacina* belongs to the family Icacinaceae. Icacinaceae were first described by Miers in 1851. At that time more than 400 species grouped in about 54 genera were recognized in this predominately pantropical family of tall rain forest, trees, shrubs, and lianas[8]. *Nothapodytes nimmoniana* (Grah.) Mabb. also known as *Mappia foetida* Meris or *Nothapodytes foetida* Sleumer belonging to family Icacinaceae is a shrubby small tree, with broad dark green leaves/flowers, distributed naturally in Westernghats of India. The plant occupies important position in the plant based anticancer drugs because of the presence ofalkaloid called campothecin (CPT). The present work describes a comparative account on the antioxidant potential of *N. nimmoniana*.

MATERIALS AND METHODS

Plant Material

Plant material of *Nothapodytes nimmoniana*(Grah.) Mabb. was collected from Siruvani hill, Coimbatore district, Tamilnadu, during the month of December 2011. The plant specimens was identified with Gambles Flora of the Presidency of Madras and the identy is confirmed with the herbarium specimen deposited in Kongunadu Arts and Science college herbarium, Coimbatore

Preparation of the Extract

Plant materials (leaf and stem) were washed with distilled water and shade dried. The dried samples were manually ground to a fine powder. The coarsely powdered parts were exhaustively extracted with methanol for 8 hr using Soxhlet apparatus. The filtrate was then evaporated to dryness under reduced pressure using rotary vaccum evaporator. The extracts were lyophilized until further use.

Chemicals

Sodium carbonate, Potassium acetate, Aluminium chloride, Gallic acid, Dimethyl Sulphoxide (DMSO), DPPH (1,1-diphenyl-2-picryl hydrazine), $ABTS^{\bullet+}$ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), Ascorbic acid, Potassium persulfate, Phosphate buffer, Potassium Ferricyanide, Trichloroacetic acid (TCA), Ferric chloride ($FeCl_3$), Ferrous sulphate (FeSO4), ferrozine, BHT (Butylated hydroxytoluene), EDTA (Ethylene diamine tetra aciticaacid), Trolex and all other chemicals with analytical grade were used.

$DPPH^{\bullet}$ Scavenging Activity

DPPH (1,1-diphenyl-2-picryl hydrazine) free radical-scavenging capabiliti es of methanolic extracts were evaluated by the method [2] Briefly, different concentrations (50, 100,150,200 and 250 mg/ml) of the extracts were pipetted out to the test tubes. 100µL of 0.2 mM alcoholic DPPH solution was added to the samples. These samples were vortexed, and incubated in dark at room temperature for 30 min. The absorbance was measured at 517 nm against blank samples. Decreased absorbance of the sample indicates $DPPH^{\bullet}$ free radical scavenging capability[5,6].

$ABTS^{\bullet+}$ Radical Scavenging Assay

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity assay was carried out using procedures described by [10]. $ABTS^{\bullet+}$ radical cations are produced by reacting ABTS 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (7 mM) and potassium persulfate (2.45 mM) and incubating the mixture at room temperature in the dark for 16 hr. The solution thus obtained was further diluted with 89% ethanol to give an absorbance of 0.700 at 734 nm. 20µL of methanolic extracts at different dilutions were added to 2 ml of ABTS and the absorbance was recorded at 734 nm after 30 minutes of incubation[1]. Trolox was used as referance standard. The percent inhibition was calculated from the following equation:

$$\text{Percentage of inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Ferrous Ion-Chelating Ability

The ferrous ion-chelating (FIC) assay reported[13] by was adopted. 2 mM FeSO4 (100µl) was mixed with different concentrations of extracts (50,100, 150,200 and 250 µl), followed by 5mM ferrozine (500 µl). Absorbance was measured at 562 nm after 10 min. The ability of extracts to chelate ferrous ions was calculated as follows:

$$\text{Percentage of inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Reducing Power Activity

Methanol extracts were determined for their reducing power by modifying the method[14]. Reaction mixtures were prepared by adding 1 ml of phosphate buffer (200m M, pH 6.6), 1 ml Potassium Ferricyanide (1%) and varying concentrations of extracts (300-700µg). After the reaction mixtures were incubated at 50°C in water bath for 20 min it was allowed to cool at room temperature (28°C) 1 ml of 10% TCA (Trichloroacetic acid) was added to each reaction mixture, and then centrifuged at 3000 rpm for 10 mins. The supernatant (2 ml) was separated in the test tube and added with 2 ml of distilled water and 0.5 ml $FeCl_3$ (1.0%), and allowed to react at room temperature and the absorbance was measured at 700 nm. Ascorbic acid was used as a standard.

Statistical Analysis

Statistical analysis of the data was performed by analysis of variance (ANOVA), using DMRT software. Statistically significance difference was denoted by probability value of $P < 0.05$. All the data were presented as Mean ± Standard Deviation (SD) for triplicates determinations.

RESULT AND DISCUSSION

DPPH

The methanolic extracts of *N. nimmoniana* significantly ($p< 0.05$) scavenge DPPH radical in a concentration dependent manner. DPPH radical scavenging activity assay assesses the capacity of the extract to donate hydrogen or to scavenge free radicals. DPPH radical is stable free radical and on reacting with an antioxidant compound which can donate hydrogen, it is reduced to diphenyl picryl hydrazine (DPPH). The switch in colour (that is, from deep-violet to light yellow) can be measured spectrophotometrically at 517 nm for various concentrations ie 50,100,150,200,250µg/ml. It was evident from the (table 1) that the methanolic extract of leaves possessed more scavenging activity(81.37 mg/ml) than methanolic extracts of stem. The results also indicate that the DPPH radicalscavenging activity increased with the increase of phenolic compound content.Ic50 value (the amount of antioxidant material required to scavenge 50 % of free radicals in the assay system) was observed as 73.67 µg/ml and 29.21 µg/ml for leaf and stem extracts respectively.

Table 1: DPPH Antioxidant Activity in Leaf and Stem Methanolic Extract of *N.nimmoniana.*

S. No	Concentration (µg/ml)	LME % of inhibition	LME IC50	SME % of inhibition	SME IC50
1	50	61.50±0.32		19.63±12.78	
2	100	63.09±0.16		19.86±0.19	
3	150	65.62±1.01	73.67	25.46±0.21	29.21
4	200	68.55±0.16		28.38±0.28	
5	250	81.37±0.25		59.02±0.24	

ABTS$^{\bullet+}$ Radical Scavenging Assay

ABTS$^{\bullet+}$ assay has been widely used to determine the free radical-scavenging activity of methanolic extract. ABTS$^{\bullet+}$ is stable free radical which dissolving methanol and their colours show characteristic absorption at 734nm. When an antioxidant scavengers the free radicals by hydrogen donation, the colours in the ABTS$^{\bullet+}$ assay solution become lighter. As presented in ABTS$^{\bullet+}$ inhibition percentage values were dose dependent where it increased in the range of the tested concentration, controls Trolox. ABTS radical cations decolourization assay showed the methanolic extract of stem was most active as the nearly fully scavenged ABTS$^{\bullet+}$.

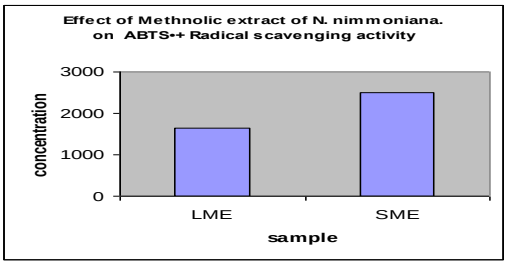

Figure 1

Table3:Effect of Methnolic extract of *N. nimmoniana.* on Ferrous Iron Chelating method

S. No	Concentration (µg/ml)	% of activity(± SD) SME	LME	Standard EDTA
1	250	56.6±0.43 [a]	58.38±0.12 [a]	56.28[a]±0.19
2	300	77.09±0.16 [b]	80.75±0.14 [b]	71.55[b]±0.33
3	350	81.45±0.12 [c]	89.15±0.05 [c]	83.46[c]±0.20
4	400	81.49±0.54 [c]	90.81±0.45 [d]	93.19[d]±0.21
5	450	95.26±1.17 [d]	96.18±0.09 [e]	96.69[e]±0.15

Ferrous Ion- Chelating Ability

Actually, hydroxyl radical can be generated by the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$), in which iron participate as a catalyst in body. The super oxide radical participates in Haber-Weiss reaction ($\cdot O_2^- + H_2O_2 \rightarrow O_2 + \cdot OH + OH^-$) which combines a Fenton reaction and the reduction of Fe^{3+} by superoxide, yielding Fe^{2+} and oxygen ($Fe^{3+} + \cdot O_2^- \rightarrow Fe^{2+} + O_2$). As ferrous ion and other transition metal ions can catalyze the oxidation in body, it is significant to investigate the metal chelating activity of an antioxidant. The ferrous ion chelating activity of methanolic leaf (96.17) extracts of *N. Nimmoniana* was observed to be high when compared with the methanolic stem extracts (81.49 ± 0.54).

Reducing Power Activity

The reducing power of methanolic extract of stem leaf were found to be increased with increasing concentration. The Reducing power of a compound may therefore serve as a significant indicator of its potential antioxidant activity in a concentration dependent manner. The reducing power of the selected methanolic extracts of *N. nimmoniana* and the reference compound, Ascorbic acid were increased steadily with increasing concentration. Among the selected methnolic extacts, the reducing power (absorbance at 700nm) of methanolic stem extract *N. nimmoniana* exhibited considerable reducing power(0.62), when compared with that of methanolic leaf extract (0.36). Hence it is suggested that methanol soluble compounds of stem might have reducing power.

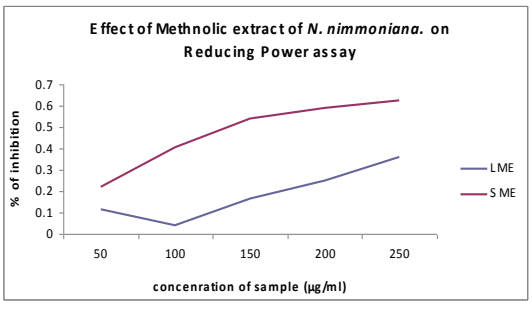

Figure 2

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